REMARKS

TELEPHONE INTERVIEW SUMMARY

Applicants appreciate the Examiner's willingness to discuss this application in a telephone interview of June 30, 2005 with the undersigned, Heeja Yoo-Warren (Reg. No. 45,495), the first named inventor (Dr. Francois Eudes) and Canadian representatives (Mary Jane McKay-Carey and Kirsten Oates). As requested by the Examiner, Applicants have amended the title to be indicative of the invention to which the claims are directed, and the abstract to meet the 150 word limit. Further, Applicants have amended claim 107 to insert the word "isolated" before the phrase "immature scutella cells" as recommended by the Examiner. Dependent claims 116, 168-178 and 180-190, which were formerly dependent upon part (d) of claim 107, have been cancelled without prejudice now that part (d) has been deleted from amended claim 107. Applicants have also amended dependent claims 124, 125, 152 and 153 to recite plants of the Pooideae subfamily to be consistent with amended claim 107 which recites Pooideae. No new matter is added by any amendment and the amendments are supported by the specification and claims as originally filed.

WITHDRAWAL OF REJECTIONS UNDER 35 U.S.C. §112, 102 AND 103

In view of Applicants' amendment filed August 13, 2004, the Office Action has withdrawn the rejections of claims 124, 125, 152, 153 and 177 under 35 U.S.C. §112, second paragraph; claim 107 under 35 U.S.C. §102(a) and (b); and claims 107-125, 127-153, 155-178 and 180-190 under 35 U.S.C. §103 (a).

CLAIM AMENDMENTS

Claims 107, 109-114, 117-125, 127-153, and 155-167 are pending herein. Applicants have amended independent claim 107 (with distinctive features in bold, underlined font):

107. A process for inducing direct somatic embryogenesis in Pooideae and rapidly regenerating fertile plants, comprising the steps of:

a) culturing **isolated immature scutella cells** of **Pooideae** in or on a culture medium comprising **auxin, cytokinin and polyamine** in amounts effective to cause **direct** formation of primary embryos **without an intervening callus stage**, at least until at least one primary embryo reaches the globular developmental stage, the **auxin being present in greater proportion than the cytokinin**;

and one of the following steps selected from:

b) culturing one or more of the primary embryos from step (a) under conditions conducive to regeneration of plantlets from the primary embryos, and culturing the primary embryo in or on a regeneration medium; or

c) culturing one or more of the primary embryos at the globular developmental stage and no longer than the coleoptilar stage from step (a) in or on a culture medium comprising **auxin, cytokinin, and polyamine** in amounts effective to cause induction of secondary embryo formation, at least until **secondary embryogenesis** is detected, the **cytokinin being present in greater proportion than the auxin**, and culturing one or more of the secondary embryos under conditions conducive to regeneration of plantlets from the secondary embryos.

Applicants clarify that the claimed invention lies in a surprisingly rapid and efficient process, which combines particular starting material, steps and conditions, even apart from the recited particular plant hormones, for regenerating fertile and non-chimeric Pooideae plants which have been known to be highly recalcitrant to prior art tissue culture techniques and genetic engineering.

Amended claim 107 recites a combination of two cycles of embryogenesis, namely direct somatic embryogenesis and secondary embryogenesis, which has been found to be surprisingly effective in regenerating a wide range of recalcitrant plant species in the Pooideae subfamily. Amended claim 107 recites that the process occurs "without an intervening callus stage," thereby avoiding problems commonly associated with prior art tissue culture methods involving indirect somatic embryogenesis (i.e., increased somaclonal variation and the loss of the ability of cells to regenerate). Amended claim 107 further recites that the embryogenic cells are cultured until at

least one embryo reaches the globular developmental stage, and step (c) clarifies that the primary embryos are cultured at the globular development stage until *de novo* embryo formation (secondary embryos) can be detected. Progression of the tissue culture steps on the basis of the developmental stage as detected by observation, rather than in accordance with a pre-determined time line, is significant. Fertile plants regenerate more rapidly than do plants with prior art tissue culture methods, and rapid regeneration makes plants less susceptible to developmental abnormalities.

Further, amended claim 107 recites the step of secondary embryogenesis, which circumvents the problem of chimeric embryos by allowing recovery of completely transformed secondary embryos from transformed sectors within a primary somatic embryo. Even if chimeric embryos are still recovered from the first cycle of secondary embryogenesis, continued cycling in the presence of a selective agent eventually results in embryos consisting entirely of transformed cells.

Amended claim 107 recites that the culture medium comprises three plant hormones (auxin, cytokinin and polyamine) and relative proportions of auxin and cytokinin, which are required to ensure the successful progression of the above recited embryogenic stages. None of the cited prior art uses all three hormones together in combination and in the recited proportions to achieve this goal.

Amended claim 107 recites initially culturing isolated immature scutella cells of Pooideae plants. The process is unique, in that tissue culture protocols of the prior art are generally more specific with regard to species, genotype, and donor tissue.

Amended claim 107 recites Pooideae for which support resides in Examples 1-3 and 5-7. Pooideae were previously considered recalcitrant to *in vitro* regeneration; however, Applicants' claimed process is believed to represent the first report of successful achievement of direct somatic embryogenesis and secondary embryogenesis in Pooideae. To the inventors' knowledge, prior art methods are generally applicable to limited species. Applicants' generic system is commercially desirable due to its capability to induce direct somatic embryogenesis and secondary embryogenesis in a wide range of Pooideae. The invention is effective independent of

the plant species, as demonstrated in Examples 1-3 and 5-7 relating to barley genotypes, wheat, durum wheat amphiploids, oat, rye, *Triticum monococum*, and *Triticum urartu*. The invention is also effective independent of genotype. As demonstrated in Examples 1-3, the invention can be applied to a variety of barley and wheat varieties including malting barley (e.g., cv Harrington), feed barley (e.g., cv AC Lacombe) and forage barley (e.g., T89043003NX), wheat (e.g., cvs AC Nanda and AC Fielder) and durum wheat amphiploids. Applicants' claimed process reduces the time required to regenerate plants using *in vitro* culture, providing green, fertile barley plants about two months earlier than the typical callus induction and regeneration approach, and green, fertile wheat plants about one month earlier than the typical callus induction and regeneration approach.

Amended claim 107 now incorporates the subject matter of claims 108 and 115 which have been cancelled without prejudice. The claim dependencies of claims 109, 111, 113, 138, 140 and 142 have been amended accordingly. Claims 124, 125, 152 and 153 have been amended to recite species of the Pooideae subfamily. Claims 116, 168-178 and 180-190 have also been cancelled without prejudice.

The amended claims are not believed to introduce any new matter. Support for the amended claims is found throughout the application and in the as-filed claims. Applicants believe that the amended claims define the invention in a manner supported by the original application, and in a manner so as to render moot certain of the rejections, as set out in greater detail below.

REJECTIONS UNDER 35 U.S.C. §102

The Office Action rejects claim 107 under 35 U.S.C. §102(b) as being anticipated by Dunstan *et al.* (1978). The Office Action states that Dunstan *et al.* discloses that cells of the scutellum of immature *Sorghum bicolor* (*Panicoideae*) were grown on MS agar medium containing 2,4-D, and that the explants proliferated into embryo-like structures characterized by structures with a "median groove" and compact white nodular tissue. The Office Action states that the explants eventually developed into plantlets by separation of the explants, and then

transferring the explants without 2,4-D. Further, the Office Action rejects claim 107 as being anticipated by Dunstan *et al.* (1979) which allegedly discloses that plantlets of *Sorghum bicolor* were formed from cultured scutellum cells without callus stage.

Applicants respectfully traverse this rejection. Applicants submit that both Dunstan *et al.* (1978 and 1979) relate to culturing of immature embryos of *Sorghum bicolor* which belongs to the Panicoideae subfamily, not to the Pooideae subfamily, as recited in Applicants' amended claim 107. Reconsideration and withdrawal of this rejection of claim 107 is thus respectfully requested.

The Office Action rejects claim 107 under 35 USC 102(b) as being anticipated by Dale. The Office Action states that Dale discloses that plantlets were produced from scutellum and shoot primordia of *Lolium multiflorum* (*Pooideae*), and that the basal culture medium was MS supplemented with 2,4-D and BAP. The Office Action states that for plant regeneration, the embryoids were cultured in MS and B5 basal media.

Applicants respectfully traverse this rejection. Applicants submit that Dale does not teach or suggest Applicants' claimed invention which combines particular steps and plant hormones to successfully regenerate plants of the Pooideae subfamily. Dale teaches culturing immature embryos of *Lolium multiflorum* using 2,4-D and BAP to produce embryo-like structures or embryoids. The embryoids were then transferred to basal MS and basal B5 media for plantlet regeneration, with the result that "half the plantlets regenerated from individual embryoids were albino" (first paragraph, page 75). It is understood by those skilled in the art that the presence of albino embryoids is a common indication of genome alteration during a callus phase. Applicants' amended claim 107 explicitly recites a process without an intervening callus stage, making plants less susceptible to developmental abnormalities and producing fertile and non-chimeric plants. Elimination of a callus stage through direct somatic embryogenesis advantageously avoids undesirable somoclonal variation.

Further, Applicants' amended claim 107 recites two cycles of embryogenesis, namely direct somatic embryogenesis and secondary embryogenesis, which lead to formation of primary embryos without an intervening callus stage and secondary embryos, respectively. The

secondary embryos are then cultured to regenerate plantlets. Dale does not mention direct somatic embryogenesis or progression of the tissue culture steps on the basis of the developmental stage of the cultured cells as recited in Applicants' amended claim 107. Successful progression of the two cycles of embryogenesis is accomplished in Applicants' invention by cultivating the cells in a culture medium containing three plant hormones (auxin, cytokinin and polyamine), as recited in Applicants' amended claim 107. Dale does not mention a combination of all three plant hormones or any polyamine. The combination of particular cycles of embryogenesis, culture medium containing these three plant hormones, and the advantages arising from same, are thus not anticipated by Dale. Reconsideration and withdrawal of this rejection of claim 107 is thus respectfully requested.

REJECTIONS UNDER 35 U.S.C. §103

The Office Action rejects claims 107-125, 127-153, 155-178 and 180-190 under 35 USC 103(a) as being unpatentable over Dale in view of Mantell *et al.*, Sargent *et al.* and Nehra *et al.* The Office Action states that:

> Dale teaches a method of regenerating plants from scutella cells. Dale further teaches that the best culture initiation medium for *Lolium* is the medium with 2 mg/l 2,4-D and 0.2 mg/l BAP for the regeneration of plants (Summary and page 76). Dale also discloses three types of tissue proliferation root, shoot and scutellar. The scutellar proliferation formed nodules on the surface which were embryo-like. These embryo-like structures were transferred to a basal MS and basal B5 for plant regeneration (page 75).
>
> Dale does not teach culturing the scutella cells in medium containing different concentration of auxin, cytokinin and polyamine. Dale also does not teach introduction of foreign DNA to the scutella cells or primary embryo.
>
> Mantell *et al.* disclose that it is well known in the art to experiment with varying degrees of growth regulators to optimize the performance of organogenesis or embryogenesis cultures of different plant species (page 142).
>
> Sargent *et al.* disclose that sorghum line TAM422 and all three sugarcane cultivars (Q117, H56752, and Q96) increase in somatic embryogenesis with the addition of polyamine to the culture media (page 454). Plant regeneration in sorghum or sugarcane all benefitted from the addition of putrescine, spermidine or spermine.

> Nehra *et al.* have shown that foreign DNA of cereals can be transformed into scutella cells of wheat and barley by particle bombardment (column 3, lines 33-37 and Figs. 1A and 1B). This method can also be advantageous for Graminaceae crop species such as corn, rice, oats, sorghum, millets and grasses (column 3, lines 39-45).
>
> It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the culture medium of Dale by supplementing with polyamine as taught by Sargent *et al.* and introducing the scutella tissues with foreign DNA as taught by Nehra *et al.* There would have been a reasonable expectation of success given that Sargent *et al.* concluded that the application of polyamine to sorghum and sugarcane provided a stimulus for somatic embryogenesis and increased plant regeneration (page 455). One of ordinary skill in the art would have been motivated to introduce foreign DNA to the scutella as shown by Nehra *et al.* because it would significantly improve the plant to certain herbicides, pest and diseases and new trait (column 1, lines 44-50). The references do not specifically teach adding growth hormones in the amounts claimed by Applicants. The amount of specific growth hormones in the medium is clearly a result effective parameter that a person of ordinary skill in the art would routinely optimize.

The Office Action further states that:

> it would have been obvious to one or ordinary skill in the art to modify the method of Dale by optimizing the growth regulator concentration as shown by Mantell *et al.* and Sargent *et al.* to induce direct somatic embryogenesis in *Panicoideae* and *Pooideae*, introducing foreign DNA as taught by Nehra *et al.*, and finally regenerating the embryos into plantlets.

Applicants respectfully traverse this rejection. Since claims 108, 115, 116, 168-190 have been cancelled without prejudice, it is understood that this rejection applies to claims 107, 109-114, 117-125, 127-153 and 155-167. The Office Action has selected Dale as the primary reference upon which all claim rejections have been based. However, Applicants submit that one skilled in the art, looking for a solution to the problem of regenerating fertile and non-chimeric Pooideae plants which have been known to be highly recalcitrant to prior art tissue culture techniques and genetic engineering, would not look to Dale or any of the cited prior art for any solution. Applicants have summarized the deficiencies of Dale as set out above. Further, the Office Action has combined the teachings of Dale with use of polyamine in Sargent *et al.*, with the basis of this combination being that polyamine can be used to supplement the medium of Dale to induce somatic embryogenesis and increase plant regeneration. Applicants note the

Office Action's statement that "absent some demonstration of unexpected results from the claimed parameters, this optimization of plant hormones amount would have been obvious at the time of Applicants' invention." Applicants believe that this is not a fair summary of Applicants' invention as now claimed in amended claim 107, since it ignores many other specific conditions of steps recited in the claims not found alone or in combination in the cited prior art.

Applicants submit that Sargent *et al.* teaches a method of regenerating plants from somatic embryogenic callus of sorghum and sugarcane using media supplemented with polyamines. Sorghum and sugarcane belong to the Panicoideae subfamily, not to the Pooideae subfamily, as recited in Applicants' amended claim 107. Further, the phrase "direct somatic embryogenesis" as used in Applicants' amended claim 107 is explicitly defined as meaning a form of embryogenesis wherein embryos develop from vegetative cells without an intervening callus stage (specification page 14, lines 28-29). Sargent *et al.* explicitly states that "sorghum and sugarcane plant regeneration was significantly increased following the addition of either putrescine, spermidine or spermine to the embryogenic callus induction medium" (paragraph 3.2, page 454). "The application of free-polyamines to sorghum and sugarcane explants can increase plant regeneration, while providing conditions for improved induction and growth of embryogenic callus" (third paragraph, page 455). Polyamine is thus used in Sargent *et al.* as a supplement in a callus induction medium in a different plant subfamily (Panicoideae), which teaches away from Applicants' claimed invention.

Applicants submit that Nehra *et al.* teaches culturing of scutella from immature zygotic embryos to produce somatic embryos which are regenerated into plantlets. Nehra *et al.*'s method which teaches use of auxin (2,4-D) and vitamin-free casamino acid "enriches the growth of embryogenic callus, but also expedites the process of somatic embryo development" (col. 5, lines 12-14). Reference is made to callus in Examples 1-5 and Figures 2B, 3E and 3H-J. Nehra *et al.* states that "distinct nodular embryogenic callus (Fig. 3E) developed on almost all isolated scutella within a week which eventually developed into a prolific mass of mature somatic embryos in 3 weeks from culture inititation" (col. 13, lines 31-35). Applicants' invention proceeds directly without an intervening callus stage as recited in amended claim 107.

Nehra *et al.* teaches culturing of scutella for 2-5 days prior to transformation with foreign DNA since the embryogenic callus formed by 4-5 days makes it more difficult to introduce the DNA into the cells. Nehra *et al.* states that higher rupture pressures are required to penetrate embryogenic callus (col. 9, lines 42-47). In contrast, Applicants' invention avoids the need for higher rupture pressure, the consequent risk of cell damage, and failure of transformation. Applicants' method proceeds without an intervening callus stage; thus, transformation can be conducted prior to development of the primary embryo under conditions conducive to direct somatic embryogenesis, but may occur anywhere from about zero to five days after commencement of tissue culture (paragraph bridging pages 32-33 of the as-filed specification). Nehra *et al.* did not recognize the enormous advantage to be gained by eliminating callus to achieve better transformation of scutella. Applicants submit that Mantell *et al.* provides very general teachings, failing to disclose any details of optimizing any medium, its composition, or any amounts.

Applicants submit that the combination of Dale's medium containing 2,4-D and BAP to produce embryo-like structures or embryoids, with Sargent *et al.*'s polyamine to promote callus growth in a different plant subfamily and Nehra's method of introducing foreign DNA into scutella tissues using high rupture pressures to penetrate embryogenic callus, does not even equate to the features recited in Applicants' amended claim 107. It is not seen how this combination would work, or how the teachings would be combined or modified to arrive at Applicants' claimed invention. Applicants' claimed invention combines particular starting material, steps and conditions, even apart from the recited particular plant hormones, for regenerating Pooideae plants. The question under 35 U.S.C. §103 is not whether the differences themselves would have been obvious, but whether the claimed invention as a whole would have been obvious. Applicants submit that it is improper to reject any of the claims under 35 U.S.C. §103. A *prima facie* case of obviousness has not been established.

In summary, none of the cited references taken alone or in any combination renders obvious any of the claims of the present application. No combination of any of the cited prior art is warranted, since none of the references show the features of the invention as claimed in the

present application. Even if a *prima facie* case of obviousness exists (which Applicants argue does not), the references do not equate to even the features of Applicants' amended claim 107 or its dependent claims. Withdrawal of this rejection of claims 107, 109-114, 117-125, 127-153 and 155-167 is respectfully requested.

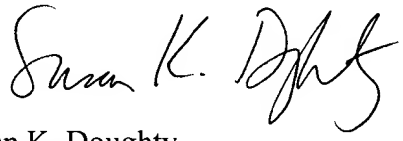
Conclusion

Applicants have complied with all requirements stated by the Examiner in the telephone interview. In view of the foregoing arguments and amendments, it is submitted that this case is in condition for allowance, and passage to issuance is respectfully requested. If there are any outstanding issued related to patentability, the courtesy of a telephone interview is requested, and the Examiner is invited to call to arrange a mutually convenient time.

This response is accompanied by a Petition for Extension of Time (two months) and the requisite fee in the amount of $450.00 as required under 37 C.F.R. 1.17.

It is believed that this response does not necessitate the payment of any additional fees under 37 C.F.R. 1.16-1.17. If the amount submitted is incorrect, however, please charge any deficiency or credit any overpayment to Deposit Account No. 07-1969.

Respectfully submitted,



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